

Attorney Docket No.: RU-0175
Inventors: Lam, Eric
Serial No.: 10/009,054
Filing Date: April 29, 2002
Page 2

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

Claim 1 (currently amended): A DNA construct for integration of heterologous DNA segments into genomes within cells, the DNA construct comprising termini having disposed therebetween a heterologous DNA ~~segments~~ segment comprising:

a) a pair of DNA substrates for a selected transposase, having disposed therebetween:

i) a first cloning site and a second cloning site for insertion of one or more additional DNA segments, wherein the first cloning site and the second cloning site have disposed therebetween a positive selection gene encoding a gene product that confers to the cells a selectable phenotype comprising resistance to a positive selection agent that is deleterious or lethal to cells having genomes in which the DNA construct has not integrated; and

ii) a negative selection gene disposed between one of the DNA substrates for the selected transposase and either the first cloning site or the second cloning site, but not between the first cloning site and the second cloning site, the negative selection gene conferring to the cells a selectable phenotype comprising susceptibility to a negative selection agent, to which cells having genomes in which the DNA construct has not integrated are not susceptible; and, optionally

b) a detectable marker gene encoding a detectable gene product, the detectable marker gene being operably inserted in

Attorney Docket No.: RU-0175
Inventors: Lam, Eric
Serial No.: 10/009,054
Filing Date: April 29, 2002
Page 3

the DNA construct relative to one of the DNA substrates for the selected transposase such that, upon excision of the DNA construct from a genome by the action of the transposase, the detectable gene product is no longer detectable.

Claim 2 (original): The DNA construct of claim 1, wherein the termini comprise at least one cloning site.

Claim 3 (original): The DNA construct of claim 1, wherein the termini comprise *Agrobacterium* tDNA left and right borders.

Claim 4 (original): The DNA construct of claim 1, wherein the DNA substrate for a selected transposase is selected from the group consisting of: a maize *Ds* element; a maize *dSpm* element, a maize *rdt* element, a maize *Mn* element, a maize *Tam2* element, a snapdragon *Tam4* element and a *Drosophila* *P* element.

Claim 5 (original): The DNA construct of claim 4, wherein the DNA substrate for a selected transposase is a maize *Ds* element and the selected transposase is a maize *Ac*-dependent transposase.

Claim 6 (original): The DNA construct of claim 1, wherein either or both of the first and second cloning sites is a polylinker.

Claim 7 (original): The DNA construct of claim 1, wherein the positive selection gene confers resistance to a selection agent selected from the group consisting of antibiotics and herbicides.

Attorney Docket No.: RU-0175
Inventors: Lam, Eric
Serial No.: 10/009,054
Filing Date: April 29, 2002
Page 4

Claim 8 (original): The DNA construct of claim 7, wherein the positive selection gene confers resistance to phosphinothricin herbicides.

Claim 9 (original): The DNA construct of claim 1, wherein the negative selection gene encodes a gene product that converts an innocuous substance to a substance that is deleterious or lethal to the cells.

Claim 10 (original): The DNA construct of claim 9, wherein the negative selection gene is a *CodA* gene.

Claim 11 (original): The DNA construct of claim 1, wherein the detectable marker gene encodes a detectable gene product selected from the group consisting of: β -glucuronidase, β -galactosidase, chloramphenicol acetyl transferase, luciferase, green fluorescent protein, alcohol dehydrogenase and a transcription factor.

Claim 12 (original): The DNA construct of claim 11, wherein the detectable marker gene encodes β -glucuronidase.

Claim 13 (original): The DNA construct of claim 1, wherein the termini comprise *Agrobacterium* tDNA right and left borders, the DNA substrate for a selected transposase comprises *Ds* substrates for a maize *Ac*-dependent transposase, the positive selection gene encodes phosphinothricin acetyltransferase, the negative selection gene encodes cytosine deaminase, the detectable marker gene encodes β -glucuronidase and the first and second cloning sites are polylinker sequences.

Attorney Docket No.: **RU-0175**
Inventors: **Lam, Eric**
Serial No.: **10/009,054**
Filing Date: **April 29, 2002**
Page 5

Claim 14 (original): The DNA construct of claim 1 wherein one or more of the positive selection gene, negative selection gene and detectable marker gene is a chimeric gene comprising a coding sequence operably linked to one or more heterologous promoters.

Claim 15 (original): The DNA construct of claim 14, wherein the promoter is selected from the group consisting of constitutive promoters, inducible promoters and tissue-specific promoters.

Claim 16 (original): The DNA construct of claim 14, wherein the chimeric gene comprises a plurality of promoters.

Claim 17 (original): The DNA construct of claim 14, wherein the promoter is a cauliflower mosaic virus 35S promoter.

Claim 18 (original): The DNA construct of claim 1, which comprises additional cloning sites disposed between the first cloning site and the second cloning site for insertion of one or more additional DNA segments, the additional cloning site being disposed relative to the positive selection gene so as not to interfere with the conferring of the selectable phenotype.

Claim 19 (original): The DNA construct of claim 1, wherein the detectable marker gene in its entirety is disposed between one of the DNA substrates for a selected transposase and the terminus closest thereto.

Claim 20 (original): The DNA construct of claim 1, wherein one of the DNA substrates for a selected transposase is located within the detectable marker gene in a manner that does not

Attorney Docket No.: RU-0175
Inventors: Lam, Eric
Serial No.: 10/009,054
Filing Date: April 29, 2002
Page 6

disrupt operability of the detectable marker gene unless the DNA substrate is acted upon by the selected transposase.

Claim 21 (original): The DNA construct of claim 20, wherein the one of the DNA substrates for a selected transposase is located between the promoter and the coding sequence of the detectable marker gene.

Claim 22 (original): The DNA construct of claim 1, operably inserted into a vector for transforming a cell.

Claim 23 (original): The DNA construct of claim 22, wherein the cell is a plant cell and the vector is an *Agrobacterium* vector.

Claim 24 (previously presented): The DNA construct of claim 1, adapted for integrating a heterologous DNA segment at a pre-determined location of a genome, wherein the adaptation comprises inserting a first targeting segment in the first cloning site and a second targeting segment in the second cloning site, each targeting segment comprising a DNA sequence homologous to sequences in the genome comprising or flanking the pre-determined location, the targeting segments enabling the DNA construct to integrate into the genome at the pre-determined location by homologous recombination.

Claim 25 (previously presented): A method for inserting a heterologous DNA molecule into a pre-determined location on a plant genome, which comprises:

Attorney Docket No.: **RU-0175**
Inventors: **Lam, Eric**
Serial No.: **10/009,054**
Filing Date: **April 29, 2002**
Page 7

a) transforming a sample of plant cells containing the genome with the DNA construct of claim 24, to produce a substrate-transformed cell line;

b) transforming an equivalent sample of plant cells with a gene encoding a transposase that specifically acts on the DNA substrates in the DNA construct, to produce a transposase-transformed cell line;

c) regenerating fertile organisms from each of the transformed cell lines;

d) crossing the substrate-transformed line with the transposase-transformed line to produce F1 progeny;

e) self-pollinating the F1 progeny to produce F2 progeny; and

f) growing the F2 progeny in the presence of the positive selection agent and the negative selection agent, progeny plants comprising the heterologous DNA inserted into the pre-determined location on the plant's genome being capable of surviving in the presence of both the positive selection agent and the negative selection agent.

Claim 26 (previously presented): The method of claim 25, which further comprises selecting a substrate-transformed cell line comprising one copy of the DNA construct per cell.

Claim 27 (original): A kit for inserting a heterologous DNA molecule into a pre-determined location on a plant genome, which comprises a container containing the DNA construct of claim 24 and instructions for using the DNA construct to insert a

Attorney Docket No.: **RU-0175**
Inventors: **Lam, Eric**
Serial No.: **10/009,054**
Filing Date: **April 29, 2002**
Page 8

heterologous DNA molecule into a pre-determined location on a plant genome.

Claim 28 (previously presented): The kit of claim 27, which further comprises a DNA construct having a gene encoding a transposase that specifically acts on the DNA substrates in the DNA construct.

Claim 29 (previously presented): A method for activation tagging of a plant genome to create variants displaying a desired phenotype, which comprises:

- a) transforming a sample of plant cells containing the genome with the DNA construct of claim 1 or claim 24, wherein the DNA construct further comprises a transcriptional promoter to transactivate expression of a plant gene near the site of insertion of the DNA construct in the genome, to produce a substrate-transformed cell line;

- b) transforming an equivalent sample of plant cells with a gene encoding a transposase that specifically acts on the DNA substrates in the DNA construct, to produce a transposase-transformed cell line;

- c) regenerating fertile organisms from each of the transformed cell lines;

- d) crossing the substrate-transformed line with the transposase-transformed line to produce F1 progeny;

- e) self-pollinating the F1 progeny to produce F2 progeny which are activation tagged; and

- f) growing the F2 progeny under conditions pre-determined to select for the desired phenotype in the plant.

Attorney Docket No.: RU-0175
Inventors: Lam, Eric
Serial No.: 10/009,054
Filing Date: April 29, 2002
Page 9

Claim 30 (original): The method of claim 29, wherein in the DNA construct, the one of the DNA substrates for a selected transposase most proximal to the 3' end of the construct is located between the promoter and the coding sequence of the detectable marker gene.

Claim 31 (previously presented): A kit for activation tagging of a plant genome to create variants displaying a desired phenotype, which comprises the DNA construct of claim 1 or claim 24, wherein the DNA construct further comprises a transcriptional promoter, and instructions for using the construct to perform activation tagging.

Claim 32 (previously presented): The kit of claim 31, which further comprises a DNA construct having a gene encoding a transposase that specifically acts on the DNA substrates in the DNA construct.